Claims

1.   A method of identifying a B cell carrying a surface immunoglobulin molecule
       having a binding site for an antigen of interest comprising

       (a) contacting a sample putatively containing said B cell

       (aa)   with the antigen of interest wherein said antigen is labeled with
               a first label; and

       (ab)   with a receptor specifically binding to said surface
               immunoglobulin molecule wherein said receptor is labeled with
               a second label; and

       wherein said first label, when being brought into a spatial proximity of

       between 10 and 100 Angstrom with said second label emits a detectable

       signal upon activation of said second label by an external source; and

       (b) assessing the presence of said detectable signal, wherein said presence

       is, in turn, indicative of the B cell carrying a surface molecule having a

       binding site for the antigen of interest.

2.   The method of claim 1, wherein said B cell is an autoreactive B cell.

3.   The method of claim 1 or 2 wherein said surface immunoglobulin molecule is
       an IgD, an IgE, an IgM or an IgG.

4.. The method of any one of claims 1 to 3, wherein said B-cell is a naïve, IgD-positive B-cell.

5. The method of any one of claims 1 to 4, wherein said antigen of interest is selected from the group consisting of

(a) auto-antigens

(b) allergens; and

(c) immunoglobulins

6. The method of any one of claims 1 to 5, wherein said sample is a sample of essentially purified B cells.

7. The method of any one of claims 1 to 6, wherein said first label is a fluorophore or fluorochrome.

8. The method of any one of claims 1 to 7, wherein said second label is a fluorophore or fluorochrome.

9. The method of any one of claims 1 to 8, wherein said receptor is an antibody or a fragment or derivative thereof.

10. The method of claim 9, wherein said antibody is directed against the Fc-part of the surface immunoglobulin molecule.

11. The method of claim 10, wherein said antibody is an anti-idiotypic antibody, wherein said anti-idiotypic antibody does not interfere with the binding site to the antigen.

12. The method of any one of claims 1 to 11, further comprising the step of isolating identified B-cells.

13. The method of claim 12, further comprising the step of cloning VH- and VL-domains from identified B cells.

14. The method of claim 13, further comprising the steps of

(a) introducing mutations in the sequences encoding said VH- and/or VL-domain/s of at least one of said B cells, wherein said mutations result in amino acid substitutions and wherein the number of mutations ranges from one to thirty, such that one or more modified antibodies is/are obtained; and/or

(b) shuffling a repertoire of V-domains to the VH- or VL-domains of said B cells, such that one or more modified antibodies is/are obtained; and/or

(c) grafting at least one CDR of at least one of the cloned VH- and/or VL-domain/s of said B cells into the corresponding position/s of the variable regions of a first antibody library, such that a second antibody library is obtained; and

(d) subjecting the resulting modified antibody/ies and/or antibody library to further selection on the antigen or parts thereof using a biological display system.

15.   The method of claim 13 or 14, further comprising the step of expressing at least one of said V-domains in an expression system.

16.   The method of any one of claims 13 to 15, further comprising the step of generating antibodies or fragments or derivatives from said V-domains.

17.   The method of claim 16, further comprising the steps of rearranging possible combinations of different VH and VL domains.

18.   The method of any one of claims 13 to 17, wherein the VH and VL domains are specific for CD28.

19.   The method of claim 18, wherein the VH- and/or VL-domains

       (a) comprise (an) amino acid sequence(s) selected from the group consisting of SEQ ID Nos: 78, 80, 82, 84, 86 and 88; and/or

       (b) are encoded by (a) nucleic acid sequence(s) comprising sequences selected from the group consisting of SEQ ID NOs: 60, 61, 79, 81, 83, 85, 87 and 89.

20.   The method of claim 16 to 19, wherein said derivatives are bispecific antibody constructs or single chain antibodies.

21.   The method of any one of claims 16 to 20, wherein said derivatives comprise at least one binding site specific for CD28.

22. The method of claim 21, wherein said derivatives

    (a) comprise the amino acid sequence as set forth in SEQ ID NO: 76; and/or

    (b) are encoded by a nucleic acid sequence comprising the sequence as set forth in SEQ ID NO: 77.

23. The method of any one of claims 1 to 22, further comprising an assay for antibody evaluation.

24. The method of claim 23, wherein said evaluation assay is a binding assay.

25. The method of claim 24, wherein said binding assay is an ELISA or a FACS based binding assay.

26. An antibody generated by the method of any one of claims 1 to 25, which is specific for human CD28.

27. An antibody generated by the method of any one of claims 1 to 26, wherein said antibody

    (a) comprises (an) amino acid sequence(s) selected from the group consisting of SEQ ID NOs: 76, 78, 80, 82, 84, 86 and 88; and/or

    (b) is encoded by (a) nucleic acid sequence(s) comprising sequences selected from the group consisting of the SEQ ID NOs: 60, 61, 77, 79, 81, 83, 85, 87 and 89.

28.   A device for assessing the presence of a detectable signal as defined in claim

1, wherein said device comprises a closed system for the detection laser-

beam and a catcher tube, and wherein the B cell of interest can be collected

as a single cell by means of an electrochemical device which is triggered by

an electric signal generated by the FACS device, wherein the electrochemical

device moves the nozzle of the steady catcher tube liquid stream for a

programmed time over a collecting tube, microtiter plate or other container

after a B cell is sorted.